

Illuminating next-generation brain therapies

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A clinical trial inspired and guided by optogenetics experiments in rodents reports the outcome of targeted transcranial magnetic stimulation in patients suffering from cocaine addiction.

Addiction is a poorly understood and heavily stigmatized disease. Substance abuse— and dependence-related conditions afflict 1 in 20 people worldwide, tear individual lives and families apart, and cause hundreds of billions of dollars a year in crime, loss of productivity and health care costs in the US alone¹. Potential treatments are complicated by side effects and inconsistent efficacy. New ideas are needed.

Technologies in basic neuroscience research have made substantial strides in recent years, including manipulations of activity patterns targeted to defined cells and connections in the brains of behaving animals (reviewed in ref. 2). These optogenetic interventions, in gain- or loss-of-function forms, can be delivered over any acute or chronic timescale from several milliseconds to many days or more^{3–5} and can also operate over many spatial scales ranging from single neurons to broad regions of the adult mammalian brain^{2,3}. The most widely used version of this approach has not been directly translated to the clinical setting at present, as it entails brain delivery of microbial opsin genes via viral vectors, along with fiber optic neural interfaces². Optogenetics has nonetheless made its presence felt beyond basic science discovery in the framing of clinical thinking and guiding of clinical interventions that use knowledge obtained from optogenetics experiments. Illustrating this bench-to-bedside influence, the first optogenetics-guided clinical trial has recently emerged from an Italian group that

used optogenetic discoveries to design and test a targeted treatment for cocaine addiction⁶.

The specific clinical hypothesis of Terraneo *et al.*⁶ arose directly from an experiment by Chen *et al.*⁷ in 2013. Rats that compulsively seek cocaine have been found to have lower activity in an area of the medial prefrontal cortex known as the prelimbic cortex than non-drug-seeking rats. When Chen *et al.*⁷ optogenetically stimulated prelimbic cortex in compulsive rats, their cocaine-seeking behavior decreased (Fig. 1). Terraneo *et al.*⁶ adapted this approach to the clinical setting using a technique called transcranial magnetic stimulation (TMS). This noninvasive brain stimulation modality, approved for treatment of major depression in the US and in Europe⁸, employs strong, rapidly changing (millisecond timescale) magnetic fields arising from coiled wires positioned outside the head, which in turn induce electrical responses inside the brain. Depending on the design of the magnetic coils, the resulting stimulation can be targeted to a focal centimeter-scale location on or near the brain surface (Fig. 1). When applied in trains of repetitive (rTMS) pulses at around 5–10 Hz, TMS is believed to induce stable potentiation-type plasticity in the targeted circuit elements; this targeting and plasticity could have the effect of modifying activity in cells, projections or brain-wide networks, depending on the protocol.

Although the rat brain is hundreds of times smaller than the human brain, with noteworthy differences in shape and complexity, many key brain regions appear to function similarly across species (Fig. 1). For example, the rodent prelimbic cortex has been linked to high-level cognitive control of behavior, with likely functional homologs in humans, including the dorsolateral prefrontal cortex (DLPFC). The human DLPFC, which is positioned superficially in the human brain, is accessible by TMS and can be precisely targeted across

individuals using magnetic resonance imaging scans to map its location and connectivity before treatment.

Terraneo *et al.*⁶ recruited 32 cocaine-addicted individuals to participate in this open study. Half of the participants were randomly allocated to rTMS therapy (40 trains of pulses to DLPFC at 15 Hz, 13 min total) and the other half were allocated to a standard drug treatment (a cocktail of medications that have been used to treat symptoms of cocaine withdrawal). Participants in both groups were treated in an outpatient clinic daily for the first 5 d and subsequently once a week for 3 more weeks; rTMS- and control-treated individuals spent similar amounts of time with the clinical team at each visit. After the initial month of treatment, individuals were given the option to switch to the opposite treatment arm and were followed for a further 2 months.

Although the study was brief, 69% of individuals in the rTMS-treated group remained drug-free during the initial treatment phase, compared with 19% of the control group (as tracked by urine drug tests). The rTMS group also reported significantly less cocaine craving. The difference between groups emerged early during the treatment: by day 12, 50% of the control group had already relapsed, whereas not a single member of the rTMS group had relapsed. A marker of naturalistic practicality was that no rTMS patients dropped out during the first month of treatment, whereas three participants were lost from the control group. The encouraging outcomes persisted during the second stage of the study, in which ten patients in the control group chose to switch to rTMS therapy. Eight of these individuals had relapsed previously on the pharmacological therapy, but, during the subsequent rTMS treatment, only three of the ten tested positive for cocaine.

A conclusion of the study, concordant with the prior rodent experiments, was that stimulation of the DLPFC may suppress the compulsion

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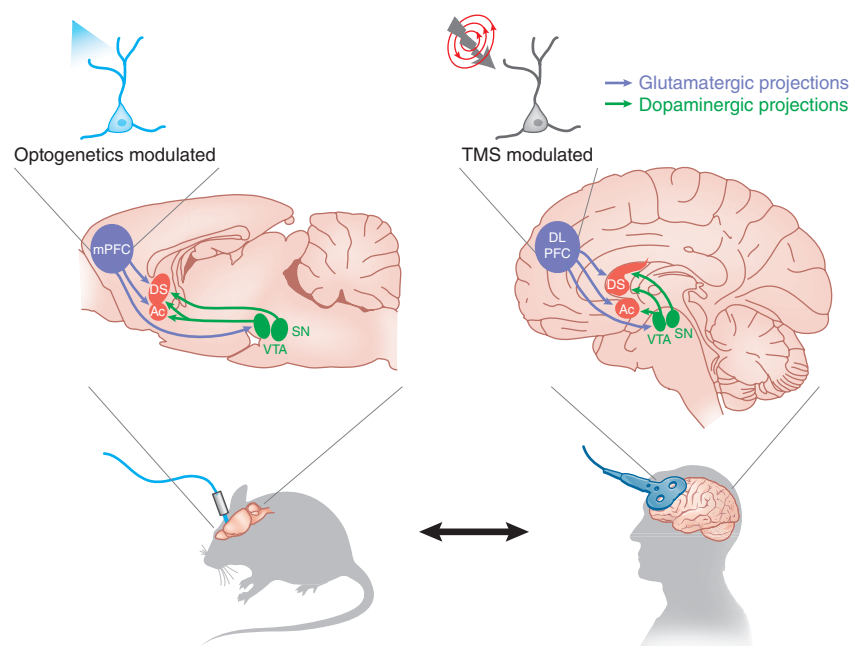


Figure 1 A clinical trial for patients with cocaine addiction guided by rodent optogenetics. Optogenetic stimulation of the medial prefrontal cortex (mPFC) with a channelrhodopsin (ChR) suppresses compulsive cocaine seeking in rats⁷. Terraneo *et al.*⁶ found that rTMS of the DLPFC appeared to prevent cocaine use relapse in cocaine-addicted patients. The mechanism of this therapeutic effect may include modulated activity in subcortical reward circuitry involving the dopaminergic midbrain (ventral tegmental area (VTA) and substantia nigra (SN)) and the striatum (dorsal striatum (DS) and nucleus accumbens (Ac)).

of cocaine-addicted subjects to seek out cocaine, further supporting the hypothesis that DLPFC hypoactivity can be a pathophysiological feature of the disorder. This result also lends support to the concept that the prefrontal cortex exerts top-down control over the brain's reward circuitry through modulation of striatal dopamine signaling; the exact mechanism by which such an effect can occur is also the subject of investigation with optogenetics and other methods (for example, ref. 9).

The authors point out some of the key limitations of the study, including its brevity and open design. To evaluate therapeutic effects, a placebo control arm (in which patients believe they are receiving rTMS but are not) is important, although in practice this may be difficult, as patients often report mild discomfort or other sensations during genuine rTMS. In the present design, the control arm received pharmacological therapy (not given to the experimental arm), with potential side effects that could influence compliance. The authors also acknowledge a significant difference in age between the two groups, with the rTMS group being, on average, 6 years older than the controls; however, *post hoc* statistical analyses revealed that this difference did not account for the effect of the therapy. The authors found no difference in depression rating scales between the two groups following treatment; however,

more subtle differences in specific psychiatric symptoms (such as anhedonia, motivation, or anxiety) were not described and could be important. Finally, a characteristic to note in the study was sex bias in both cohorts (only two women in the rTMS group, and zero in the control group). Although men account for the majority of drug users, cocaine use is increasing among women, with approximately one-third of cocaine users in the United States being female¹⁰. Future studies should address these demographic and design issues, as well as extend the study duration; physicians, patients and families are all too aware of the high long-term risk of relapse associated with substance abuse and dependence.

The study by Terraneo *et al.*⁶ not only stirs new hope for principled interventional treatment of addiction, but also, more fundamentally, defines a new process for neuroscience translation, as the initial published outcome of an optogenetics-guided clinical trial. The principle is generalizable; optogenetics is on the whole a basic science discovery technology, but can also guide modifications to existing therapies even beyond TMS. For example, early optogenetic studies of parkinsonism and deep brain stimulation (DBS)-like intervention in rodents highlighted the therapeutic potential of targeting white-matter projection pathways to deliver more effective neuromodulation¹¹.

Since then, a number of optogenetic studies have confirmed the potency and specificity of such projection targeting in modulating psychiatry-related phenotypes (reviewed in ref. 12), and this general principle has provided a basic foundation for clinical teams performing fiber tract mapping with diffusion tensor imaging to guide electrode placement for human DBS in Parkinson's disease¹³ and depression¹⁴.

By analogy with precise genetic interventions (which even in the case of single-gene modifications can only exert their ultimate effects through cascades of perturbed downstream molecular elements), precise activity interventions in neural circuitry (whether electrical, optical, pharmacological or surgical) must also exert their behavioral effects through downstream effectors interacting across the brain⁹. Looking forward, clinical trials may build on basic science investigation of this natural complexity—for example, in design of optogenetically guided protocols to combine less-specific, but clinically available, neurostimulation with concomitant pharmacology to counteract inevitable off-target effects (direct non-targeted element modulation) of non-optogenetic interventions. This idea was recently demonstrated in another study of cocaine addiction¹⁵, which reported that 12-Hz optogenetic stimulation of prefrontal cortex projections to the nucleus accumbens suppressed sensitivity to a cocaine challenge in rats. Surprisingly, less-specific 12-Hz electrical DBS had no such effect unless combined with systemic administration of D1 dopamine receptor antagonists, suggesting that true off-target dopaminergic activity (that is, directly electrode-driven spurious activation of local dopaminergic projections, alongside the local desired direct target of prefrontal-to-accumbens glutamatergic projections) could be interfering with what would otherwise be a promising therapeutic strategy. Thus, two frequently used clinical treatments (DBS and pharmacotherapy) could be combined in a principled optogenetics-guided and hypothesis-driven manner for joint benefit.

A challenge for the future will be to optimize neuromodulation parameters for anatomical and temporal precision, minimally invasive access to deep brain regions, and strong safety and efficacy profiles that leverage the avalanche of insights emerging from the basic science laboratories. Meanwhile, fundamental laboratory research will continue to lay essential foundations for understanding the brain as a complex dynamical system and for probing the causal underpinnings of adaptive and maladaptive behaviors.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the [online version of the paper](#).

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Targeting PTEN interactions for Alzheimer's disease

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Depression of AMPA receptor-mediated synaptic currents and impairment of long-term potentiation, triggered by amyloid- β , are the hallmarks of Alzheimer's pathophysiology. These dysfunctions are now linked to upregulated PDZ domain-dependent PTEN translocation to spines, contributing to cognitive deficits in model mice.

Phosphatase and tensin homolog located on chromosome 10 (PTEN) is a lipid and protein phosphatase that was originally characterized as a tumor suppressor. Eventually, it became clear that PTEN regulates a diverse range of functions in the CNS. Today we know that deregulation of PTEN affects neurogenesis, neurite outgrowth, synaptogenesis, synaptic transmission and plasticity¹. Human *PTEN* mutations or conditional deletions of *Pten* in mice have provided insights into pathogenesis of various neurological disorders, such as macrocephaly, ataxia, seizures, autism and mental retardation¹.

In this issue of *Nature Neuroscience*, Knafo *et al.*² reveal a new pathway that depresses excitatory hippocampal synapses in Alzheimer's disease (AD) models. This pathway centers on PTEN, which antagonizes the activity of class I phosphatidylinositol 3-OH kinase (PI3K). PI3K synthesizes phosphatidylinositol-3,4,5-trisphosphate (PIP₃) from phosphatidylinositol-4,5-bisphosphate (PIP₂). PTEN is the main identified 3-phosphatase that can convert PIP₃ back into PIP₂ at the plasma membrane, thereby arresting PI3K-Akt pathway-dependent signaling.

A wide range of AD-related synaptic and neuronal dysfunctions have been discovered during the last few decades, ranging from

hyperactivity to depression of hippocampal and cortical circuits³. Yet the exact molecular mechanisms and the temporal profile of different cellular and network-wide changes remain elusive. Given that hyperactivity of hippocampal function occurs in humans at risk for AD⁴, synaptic depression and subsequent loss may represent the compensatory mechanisms activated by homeostatic control system in an attempt to stabilize brain function⁵. Using a combination of electrophysiological, optical, biochemical and behavior tools in various cellular and animal AD models, Knafo *et al.*² discovered that the PDZ-binding domain of PTEN is central to the well-known AD-associated postsynaptic dysfunctions.

Knafo *et al.*² took advantage of a specific pharmacological PTEN inhibitor, VO-OHpic, in adult mice. This choice was critical because *Pten* deletion during early postnatal development, even in a small subset of hippocampal neurons, causes seizures and changes in neuronal morphology⁶, and *Pten* haploinsufficiency increases neuronal excitability⁷. Intracerebroventricular delivery of VO-OHpic for 3–4 weeks ameliorated the cognitive deficits and long-term potentiation (LTP) deficits in the CA3–CA1 hippocampal pathway in mice carrying familial AD (FAD) mutations in amyloid precursor protein (APP) and presenilin-1 (PS1) proteins (APP/PS1 mice). The observed renormalization of cognitive and synaptic functions were not associated with detectable changes in APP metabolism or levels of amyloid- β (A β), a product of APP proteolytic cleavage, indicating that PTEN acts downstream of A β production.

The authors used different preparations and approaches to identify the mechanisms underlying rescue of AD-related postsynaptic dysfunctions following PTEN inhibition. Using short-term (2 h) application of aggregated A β ₄₂ in wild-type acute hippocampal slices and transient (1–2 d) overexpression of APP with FAD mutations (APP^{Swe/Lnd}) in organotypic slice cultures, the authors observed depression of basal synaptic transmission mediated by AMPA-type glutamate receptors (AMPA), confirming the results of an earlier study⁸. Both functional and structural postsynaptic changes were prevented by VO-OHpic pretreatment in cellular APP models. However, 1 month of VO-OHpic treatment did not rescue CA3–CA1 synaptic depression in slices from adult APP/PS1 mice. It is noteworthy that pretreatment with VO-OHpic consistently prevented LTP deficits in all three models, including the APP/PS1 mice treated for 3 weeks.

The lack of VO-OHpic effect on basal synaptic transmission in APP/PS1 mice is puzzling. A few possibilities may explain the inability of the PTEN inhibitor to rescue synaptic depression in APP/PS1 mice, unlike in the other preparations. First, blockade of PTEN may be effective only at the early stages of treatment owing to compensation by homeostatic mechanisms at later stages. Second, PS1 may participate in a mechanism of synaptic depression that is independent of A β and/or PTEN. For example, FAD PS1 mutations may increase an activation of the glycogen synthase kinase-3 β (GSK3 β) pathway, leading to tau hyperphosphorylation, a prominent AD-related intracellular pathology⁹. It would be important to

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